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CORPORATE INTELLECTUAL PROPERTY

To: Examiner J. Mullis Fax No.: 703-872-9666

From: Nora Stein-Fernandez Fax No.: (610) 270-5090
Phone No.: (610) 270-5044

Date: February 8, 2001

Page 1 of: 24

If you do not receive all pages or if pages are not legible, please contact Diane Halata at 610-270-5032.

Re: Serial No. 08/450,437 filed May 25, 1995
Our Docket No. P30958C2

OK to Enter
2-01

Dear Examiner Mullis:

Further to our telephone conversation yesterday, attached is a copy of the Petition to Withdraw from Issue Under 37 C.F.R. §1.313(b)(3) With Amendment of Claims filed September 22, 1999.

Also attached is a Supplemental IDS, citing a reference (in Category A) found by the EPO Search Authority.

If you have any questions, please call me at 610-270-5044.

Sincerely,

Nora Stein-Fernandez
Senior Patent Counsel

CORPORATE INTELLECTUAL PROPERTY-U.S. - UW2220
709 Swedeland Road, PO Box 1539, King of Prussia, PA 19406-0939 USA

#17E

Attorney Docket No. P30958C2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Farina et al. September 22, 1999

Serial No.: 08/450,437 Group Art Unit No.: 1711

Filed: May 25, 1995 Examiner: J. Mullis

For: QUINOLINE DERIVATIVES Batch No.: P58

Assistant Commissioner of Patents
Washington, D.C. 20231

PETITION TO WITHDRAW FROM ISSUE UNDER 37 C.F.R. §1.313(b)(3) WITH AMENDMENT OF CLAIMS

In this application, the issue fee was paid on May 25, 1999, in response to a Notice of Allowance mailed February 26, 1999. Applicants now petition to withdraw this application from issue under the provisions of 37 C.F.R. §1.313(b)(3). Applicants unequivocally submit that allowed claims 1-14 and 17-21 are unpatentable over the enclosed copy of U.S. Patent No. 4,711,890, issued December 8, 1997, not previously submitted in this application ("the '890 patent"). Approval of this petition is respectfully requested.

A Supplemental Information Disclosure Statement and PTO Form 1449 are also enclosed.

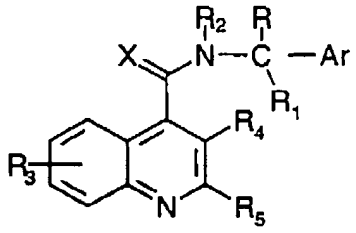
In the event such petition is accepted, Applicants submit the following claims which are believed to be outside the scope of the newly cited art. None of the claims add new matter to this application. No new search is believed necessary based upon the claims now presented. In addition, the Commissioner is hereby authorized to charge the requisite fee under 37 C.F.R §1.17(i) ($130.00) to Deposit Account No. 19-2570.

IN THE CLAIMS:

Please cancel claims 1 and 17, and add new claims 59-61, as follows:

--59. A compound, or solvate or salt thereof, of formula (I):

E1 cont



(I)

in which:

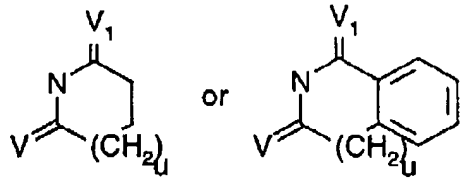
Ar is an optionally substituted phenyl group, or a naphthyl or C_{5-7} cycloalkdienyl group, or an optionally substituted single or fused ring heterocyclic group, having aromatic character, containing from 5 to 12 ring atoms and comprising up to four hetero-atoms in the or each ring selected from S, O, N;

R is linear or branched C_{1-8} alkyl, C_{3-7} cycloalkyl, C_{4-7} cycloalkylalkyl, an optionally substituted phenyl group or a phenyl C_{1-6} alkyl group, an optionally substituted five-membered heteroaromatic ring comprising up to four heteroatoms selected from O and N, hydroxy C_{1-6} alkyl, amino C_{1-6} alkyl, C_{1-6} alkylaminoalkyl, di C_{1-6} alkylaminoalkyl, C_{1-6} acylaminoalkyl, C_{1-6} alkoxyalkyl, C_{1-6} alkylcarbonyl, carboxy, C_{1-6} alkoxyxcarbonyl, C_{1-6} alkoxycarbonyl C_{1-6} alkyl, aminocarbonyl, C_{1-6} alkylaminocarbonyl, di C_{1-6} alkylaminocarbonyl, halogeno C_{1-6} alkyl; or is a group $-(CH_2)_p-$ when cyclized onto Ar, where p is 2 or 3;

R_1 is hydrogen or C_{1-6} linear or branched alkyl, or together form a $-(CH_2)n-$ group in which n represents 3, 4, or 5; or R_1 together with R forms a group $-(CH_2)_q-$, in which q is 2, 3, 4 or 5;

R_2 is hydrogen;

R_3 is hydrogen, C_{1-6} linear or branched alkyl, C_{1-6} alkenyl, aryl, C_{1-6} alkoxy, hydroxy, halogen, nitro, cyano, carboxy, carboxamido, sulphonamido, C_{1-6} alkoxycarbonyl, trifluoromethyl, acyloxy, phthalimido, amino, mono- and di-C_{1-6} alkylamino, $-O(CH_2)_r-NT_2$, in which r is 2, 3, or 4 and T is hydrogen or C_{1-6} alkyl or it forms with the adjacent nitrogen a group

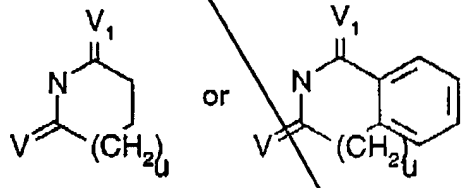


in which V and V_1 are independently hydrogen or oxygen and u is 0,1 or 2;

$-O(CH_2)_s-OW_2$ in which s is 2, 3, or 4 and W is hydrogen or C_{1-6} alkyl; hydroxyalkyl, aminoalkyl, mono-or di-alkylaminoalkyl, acylamino, alkylsulphonylamino, aminoacylamino, mono- or di-alkylaminoacylamino; with up to four R_3 substituents being present in the quinoline nucleus;

R_4 is C_{1-6} linear or branched alkyl, C_{1-6} alkenyl, aryl, C_{1-6} alkoxy, hydroxy, halogen, nitro, cyano, carboxy, carboxamido, sulphonamido, C_{1-6} alkoxycarbonyl, trifluoromethyl, acyloxy, phthalimido, amino, mono- and di-C_{1-6} alkylamino,

$-O(CH_2)_r-NT_2$, in which r is 2, 3, or 4 and T is hydrogen or C_{1-6} alkyl or it forms with the adjacent nitrogen a group



in which V and V_1 are independently hydrogen or oxygen and u is 0,1 or 2;

$-O(CH_2)_s-OW_2$ in which s is 2, 3, or 4 and W is hydrogen or C_{1-6} alkyl; hydroxyalkyl, aminoalkyl, mono-or di-alkylaminoalkyl, acylamino, alkylsulphonylamino, aminoacylamino, mono- or di-alkylaminoacylamino; with up to four R_3 substituents being present in the quinoline nucleus;

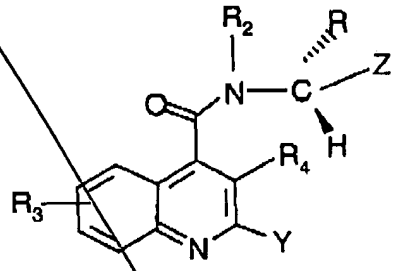
or R_4 is a group $-(CH_2)_t-$ when cyclized onto R_5 as aryl, in which t is 1, 2, or 3;

R_5 is branched or linear C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{4-7} cycloalkylalkyl, optionally substituted aryl, wherein an optional substituent is hydroxy, halogen, C_{1-6} alkoxy or C_{1-6} alkyl, or an optionally substituted single or fused ring heterocyclic group, having aromatic character, containing from

5 to 12 ring atoms and comprising up to four hetero-atoms in the or each ring selected from S, O, N;

X is O, S, or N-C≡N.

23 60. A compound, or solvate or salt thereof, of formula (Ib):



(Ib)

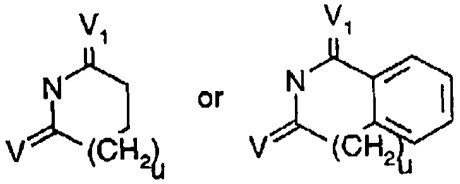
in which:

R is linear or branched C_{1-8} alkyl, C_{3-7} cycloalkyl, C_{4-7} cycloalkylalkyl, an optionally substituted phenyl group or a phenyl C_{1-6} alkyl group, an optionally substituted five-membered heteroaromatic ring comprising up to four heteroatoms selected from O and N, hydroxy C_{1-6} alkyl, amino C_{1-6} alkyl, C_{1-6} alkylaminoalkyl, di C_{1-6} alkylaminoalkyl, C_{1-6} acylaminoalkyl, C_{1-6} alkoxyalkyl, C_{1-6} alkylcarbonyl, carboxy, C_{1-6} alkoxyxcarbonyl, C_{1-6} alkoxycarbonyl C_{1-6} alkyl, aminocarbonyl, C_{1-6} alkylaminocarbonyl, di C_{1-6} alkylaminocarbonyl, halogeno C_{1-6} alkyl; or is a group $-(CH_2)_p-$ when cyclized onto Ar, where p is 2 or 3;

R_1 is hydrogen or C_{1-6} linear or branched alkyl, or together form a $-(CH_2)n-$ group in which n represents 3, 4, or 5; or R_1 together with R forms a group $-(CH_2)_q-$, in which q is 2, 3, 4 or 5;

R_2 is hydrogen;

R_3 is hydrogen, C_{1-6} linear or branched alkyl, C_{1-6} alkenyl, aryl, C_{1-6} alkoxy, hydroxy, halogen, nitro, cyano, carboxy, carboxamido, sulphonamido, C_{1-6} alkoxycarbonyl, trifluoromethyl, acyloxy, phthalimido, amino, mono- and di-C_{1-6} alkylamino, $-O(CH_2)_r-NT_2$, in which r is 2, 3, or 4 and T is hydrogen or C_{1-6} alkyl or it forms with the adjacent nitrogen a group

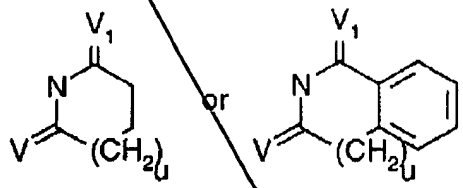


in which V and V_1 are independently hydrogen or oxygen and u is 0,1 or 2;

$-O(CH_2)_s-OW_2$ in which s is 2, 3, or 4 and W is hydrogen or C_{1-6} alkyl; hydroxyalkyl, aminoalkyl, mono-or di-alkylaminoalkyl, acylamino, alkylsulphonylamino, aminoacylamino, mono- or di-alkylaminoacylamino; with up to four R_3 substituents being present in the quinoline nucleus;

R_4 is C_{1-6} linear or branched alkyl, C_{1-6} alkenyl, aryl, C_{1-6} alkoxy, hydroxy, halogen, nitro, cyano, carboxy, carboxamido, sulphonamido, C_{1-6} alkoxycarbonyl, trifluoromethyl, acyloxy, phthalimido, amino, mono- and di-C_{1-6} alkylamino,

$-O(CH_2)_r-NT_2$, in which r is 2, 3, or 4 and T is hydrogen or C_{1-6} alkyl or it forms with the adjacent nitrogen a group



in which V and V_1 are independently hydrogen or oxygen and u is 0,1 or 2;

$-O(CH_2)_s-OW_2$ in which s is 2, 3, or 4 and W is hydrogen or C_{1-6} alkyl; hydroxyalkyl, aminoalkyl, mono-or di-alkylaminoalkyl, acylamino, alkylsulphonylamino, aminoacylamino, mono- or di-alkylaminoacylamino; with up to four R_3 substituents being present in the quinoline nucleus;

or R_4 is a group $-(CH_2)_t-$ when cyclized onto R_5 as aryl, in which t is 1, 2, or 3;

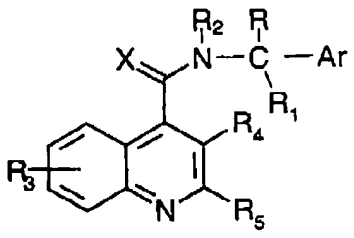
Z is phenyl or phenyl substituted by hydroxy, halogen, C_{1-6} alkoxy, C_{1-6} alkyl or Z is a single or fused ring heterocyclic group, having aromatic character, containing from 5 to 12 ring atoms and comprising up to four hetero-atoms in the or each ring selected from S, O, N or Z is C_{5-7} cycloalkdienyl; and

Y is C_{3-7} cycloalkyl, phenyl or phenyl substituted by hydroxy, halogen, C_{1-6} alkoxy, or

C_{1-6} alkyl, or Y is a single or fused ring heterocyclic group, having

aromatic character, containing from 5 to 12 ring atoms and comprising up to four hetero-atoms in the or each ring selected from S, O, N.

61. A compound, or solvate or salt thereof, of formula (I):



(I)

in which:

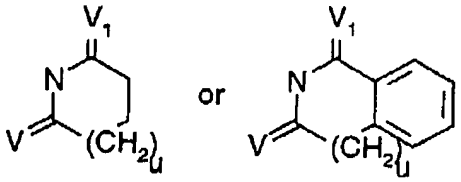
Ar is an optionally substituted phenyl group, or a naphthyl or C_{5-7} cycloalkdienyl group, or an optionally substituted single or fused ring heterocyclic group, having aromatic character, containing from 5 to 12 ring atoms and comprising up to four hetero-atoms in the or each ring selected from S, O, N;

R is linear or branched C_{1-8} alkyl, C_{3-7} cycloalkyl, C_{4-7} cycloalkylalkyl, an optionally substituted phenyl group or a phenyl C_{1-6} alkyl group, an optionally substituted five-membered heteroaromatic ring comprising up to four heteroatoms selected from O and N, hydroxy C_{1-6} alkyl, amino C_{1-6} alkyl, C_{1-6} alkylaminoalkyl, di C_{1-6} alkylaminoalkyl, C_{1-6} acylaminoalkyl, C_{1-6} alkoxyalkyl, C_{1-6} alkylcarbonyl, carboxy, C_{1-6} alkoxyxcarbonyl, C_{1-6} alkoxycarbonyl C_{1-6} alkyl, aminocarbonyl, C_{1-6} alkylaminocarbonyl, di C_{1-6} alkylaminocarbonyl, halogeno C_{1-6} alkyl; or is a group $-(CH_2)_p-$ when cyclized onto Ar, where p is 2 or 3;

R_1 is hydrogen or C_{1-6} linear or branched alkyl, or together form a $-(CH_2)n-$ group in which n represents 3, 4, or 5; or R_1 together with R forms a group $-(CH_2)_q-$, in which q is 2, 3, 4 or 5;

R_2 is hydrogen;

R_3 is hydrogen, C_{1-6} linear or branched alkyl, C_{1-6} alkenyl, aryl, C_{1-6} alkoxy, hydroxy, halogen, nitro, cyano, carboxy, carboxamido, sulphonamido, C_{1-6} alkoxycarbonyl, trifluoromethyl, acyloxy, phthalimido, amino, mono- and di-C_{1-6} alkylamino, $-O(CH_2)_r-NT_2$, in which r is 2, 3, or 4 and T is hydrogen or C_{1-6} alkyl or it forms with the adjacent nitrogen a group



in which V and V_1 are independently hydrogen or oxygen and u is 0,1 or 2;

$-O(CH_2)_s-OW_2$ in which s is 2, 3, or 4 and W is hydrogen or C_{1-6} alkyl; hydroxyalkyl, aminoalkyl, mono-or di-alkylaminoalkyl, acylamino, alkylsulphonylamino, aminoacylamino, mono- or di-alkylaminoacylamino; with up to four R_3 substituents being present in the quinoline nucleus;

R_4 is hydroxy;

R_5 is branched or linear C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{4-7} cycloalkylalkyl, optionally substituted aryl, wherein an optional substituent is hydroxy, halogen, C_{1-6} alkoxy or C_{1-6} alkyl, or an optionally substituted single or fused ring heterocyclic group, having aromatic character, containing from 5 to 12 ring atoms and comprising up to four hetero-atoms in the or each ring selected from S, O, N;

X is O, S, or N-C≡N.--.

Please amend the claims, as follows:

for each of claims 2-13, at line 1, delete "claim 1" and insert --claim 59--;

10 ~~14.~~ (Amended) A pharmaceutical composition comprising a compound of formula (I) or salt or solvate thereof, as defined in claim [1] 59, and a pharmaceutically acceptable carrier.

11 ~~15.~~ (Twice Amended) A method for the treatment and/or prophylaxis of pulmonary disorders skin disorders and itch, neurogenic inflammation and CNS disorders, convulsive disorders, epilepsy, renal disorders, urinary incontinence, ocular inflammation, inflammatory pain, eating disorders, allergic rhinitis, neurodegenerative disorders, psoriasis, Huntington's disease, and depression in mammals, which comprises administering to the mammal in need of such treatment and/or prophylaxis an effective amount of a compound of formula (I), or a solvate or salt thereof, as defined in claim [1] 59.

for each of claims 18-20, at line 1, delete "claim 17" and insert --claim

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Batch No.: P58
Group Art Unit: 1711

60--.

21. (Amended) A pharmaceutical composition comprising a compound according to claim [17] 60 or a pharmaceutically acceptable a salt or solvate thereof and a pharmaceutically acceptable carrier.

22. (Twice Amended) A method for the treatment and/or prophylaxis of pulmonary disorders skin disorders and itch, neurogenic inflammation and CNS disorders, convulsive disorders, epilepsy, renal disorders, urinary incontinence, ocular inflammation, inflammatory pain, eating disorders, allergic rhinitis, neurodegenerative disorders, psoriasis, Huntington's disease, and depression in mammals, which comprises administering to the mammal in need of such treatment and/or prophylaxis an effective amount of a compound of formula (I), or a solvate or salt thereof, as defined in claim [17] 60.

REMARKS

This paper is a combined Petition to Withdraw from Issue Under 37 C.F.R. §1.313(b)(3), as well as an Amendment with claims falling outside the scope of the newly cited art. By this amendment, new claims 59-61 are presented. Allowed claims 2-15 have been amended in order to depend from new claim 59. Allowed claims 18-22 have been amended in order to depend from new claim 60. Allowed claims 23-58 are believed to be free of the art already cited in this case, as well as the '890 patent. In addition, new claims 59-61, and amended claims 2-15 and 18-22 are believed to be patentable in view of the '890 patent. Support for the addition of new claims 59-61 is found in the specification, examples and claims as originally filed. Therefore, Applicants submit that the new claims are now patentable. No additional searching is believed required by these newly presented claims.

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9

In view of the foregoing, Applicants respectfully request the grant of this Petition to Withdraw this application from issue, and allowance of claims 2-16 and 18-61.

Respectfully submitted,

Nora Stein-Fernandez

Nora Stein-Fernandez
Attorney for Applicants
Registration No. 36,689

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CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

Diane W. Halata

Diane W. Halata
Signature

2/8/01
Date

Attorney Docket No. P30958C2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Farina et al.	8 February 2001
Serial No.:	08/450,437	Group Art Unit No.: 1711
Filed:	May 25, 1995	Examiner: J. Mullis
For:	QUINOLINE DERIVATIVES	

Assistant Commissioner for Patents
Washington, D.C. 20231

Information Disclosure Statement Under 37 C.F.R. §1.97(c)

The attached list of citations is being submitted under the provisions of 37 C.F.R. §1.56 and §1.97 in order to comply with the duty of disclosure. Their inclusion herein, however, should not be construed as an admission that any particular cited reference is effective prior art or that it discloses or renders obvious any aspect of the claimed invention.

The document cited herein is listed on the enclosed PTO Form 1449. A copy is enclosed. In addition, a copy of the European Search Report citing this document is enclosed.

This statement is being filed under the provisions of 37 C.F.R. §1.97(c), before the mailing date of a Final Office Action or before the mailing date of a Notice of

Serial No.: 08/450,437
Group Art Unit No.: 1711

Allowance, along with authorization to charge the $240.00 fee specified in 37 C.F.R. §1.17(p) to the Deposit Account No. 19-2570.

Please charge any additional fees under 37 C.F.R. §1.16 or §1.17 which may be required by this paper, or credit any overpayment, to the indicated Deposit Account.

Respectfully submitted,

Nora Stein-Fernandez

Nora Stein-Fernandez
Attorney for Applicants
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J. Med. Chem. 1993, 36, 2-10

Articles

Cyclic Peptides as Selective Tachykinin Antagonists

Brian J. Williams,* Neil R. Curtis, Alexander T. McKnight, Janet J. Maguire, Stephen C. Young, Daniel F. Veber,† and Raymond Baker

Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, England

Received September 27, 1990

Twenty homodetic cyclic peptides based on the C-terminal sequence of substance P were prepared (Table I) by a combination of solid-phase techniques and cyclizations using azide coupling procedures. Incorporation of dipeptide mimics based on substituted γ-lactams were used in some cases to restrict their conformational mobility. Five of these cyclic peptides were shown to have high tachykinin antagonist activity ($pA_2 > 6$) at NK-2 receptors (rat vas deferens). The two most potent of this series, XVII, cyclo(Gln-Trp-Phe-Gly-Leu-Met) ($pA_2 = 8.1$), and I cyclo(Gln-Trp-Phe-(R)Gly[ANC-2]Leu-Met) ($pA_2 = 6.7$), were selective for NK-2 receptors compared with the other tachykinin receptors (Table II).

The tachykinins[1] are a family of peptides which have a closely homologous carboxy terminus (Figure 1). The naturally occurring mammalian tachykinins, substance P, neurokinin A, and neurokinin B[2], exert a variety of potent actions on a number of smooth muscle and glandular tissues and in the central nervous system.[3] Although structurally similar, the endogenous tachykinins have different biological activities as a consequence of the existence of a number of receptor subclasses.[4,5] Originally the identification of receptor subtypes was based solely on the rank order of potency of the tachykinin agonists and desensitization experiments.[6] Full characterization of these receptor subclasses and, indeed, the study of the relative physiological function of the natural tachykinins have been hampered by the absence of selective antagonists. We now describe the preparation of a series of cyclic peptides which are tachykinin antagonists, some of which show a high degree of site selectivity for the NK-2 subclass of receptor.[7,8]

The approach to developing tachykinin antagonists adopted by Folkers[9] and more recently by Escher[10] and others[11] has been to modify the structure of substance P (SP) and the neurokinins systematically by introduction of D-amino acid residues (particularly with D-Trp and D-Phe). Crucial residues for antagonist activity appear to be Phe[7] and Gly[9] and antagonists such as [D-Pro[2],D-Phe[7],D-Trp[9]]SP were developed. It is difficult to determine whether these antagonists, with such a high proportion of aromatic residues, bind to the receptor in the same way as the natural hormones and whether these D-residues, for example at position 9, themselves bind to the receptor

MAMMALIAN TACHYKININS

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

NON-MAMMALIAN TACHYKININS

Eledoisin	Glp-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2
Physalaemin	Glp-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
Kassinin	Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2

Figure 1.

* Author to whom all correspondence should be addressed.
† Merck Sharp and Dohme Research Laboratories, West Point, PA 19486.

(1) Erspamer, V. The tachykinin family. *Trends Neurosci.* 1981, 267–269.
(2) Henry, J. L. In *Substance P and Neurokinins—Montreal '86*. Proceeding of the XXX International Congress of the International Union of Physiological Sciences; Henry, J. L., Couture, R., Cuello, A., Pelletier, G., Quirion, R., Regoli, D., Eds.; Springer-Verlag Publishing Co.: New York, 1987; pp xvii–xviii.
(3) Pernow, B. Substance P. *Pharmacol. Rev.* 1983, 35, 85–141.
(4) (a) Iversen, L. L.; Watling, K. J.; McKnight, A. T.; Williams, B. J.; Lee, C. M. In *Proceedings of the 4th SCI-RSC Medicinal Chemistry Symposium*; 1987, Churchill College, Cambridge. (b) Lee, C.-M., Campbell, N. J., Williams, B. J. and Iversen, L. L. Multiple binding sites in peripheral tissues and in brain. *Eur. J. Pharm.* 1986, *130*, 209–217.
(5) Quirion, R. Multiple tachykinin receptors. *Trends Neurosci.* 1985, *8*, 183–185.
(6) Lee, C. M.; Iversen, L. L.; Hanley, M. R.; Sandberg, B. E. B. The possible existence of multiple receptors for substance P. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1982, *318*, 281–287.
(7) Williams, B. J.; Curtis, N. R.; McKnight, A. T.; Maguire, J.; Foster, A.; Tridgett, R. Development of NK-2 selective antagonists. *Regul. Pept.* 1988, *22*, 189.
(8) McKnight, A. T.; Maguire, J. J.; Williams, B. J.; Foster, A. C.; Tridgett, R.; Iversen, L. L. Pharmacological specificity of synthetic peptides as antagonists at tachykinin receptors. *Regul. Peptides* 1988, *22*, 117.
(9) Folkers, K.; Horig, J.; Rosell, S.; Bjorkroth, U. Chemical design of antagonists of substance P. *Acta Physiol. Scand.* 1981, *111*, 505–506.
(10) Regoli, D.; Escher, E.; Mizrahi, J. Substance P-Structure activity studies and the development of antagonists. *Pharmacology* 1984, *28*, 301–320.
(11) Dutta, A. S.; Gormley, J. J.; Graham, A. S.; Briggs, I.; Crowcott, J. W.; Jamieson, A. Analogues of substance P. Peptides containing D-amino acid residues in various positions of substance P and displaying agonist or receptor selective antagonist effects. *J. Med. Chem.* 1986, *29*, 1163–1171.

0022-2623/93/1836-0002$04.00/0

or are responsible solely for altering backbone conformations.. These uncertainties together with the high conformational mobility normally associated with linear peptides makes interpretation of structure–activity relationships particularly difficult. Substitution of amino acids, found to be optimal in one series of peptides, may not necessarily be optimal in other series. Nevertheless antagonists which show selectivity toward the various receptor subclasses have been prepared by the careful choice of amino acid substitution.[12]

An alternative approach to obtaining tachykinin antagonists, which involves the use of conformational constraints, has recently been reported by Ward et al.[13] Incorporation of spirolactams into linear peptide sequences homologous to substance P have provided both NK-1 selective agonists and antagonists. Agonists were produced when an (*R*)-spirolactam unit was used which can be considered to be a hybrid of the (*R*)-Gly[ANC-2]Leu lactam[14] and L-Pro-Leu sequences in place of the natural Gly-Leu sequences in substance P. However, in contrast, when the (*S*)-spirolactam unit was incorporated, antagonist activity was observed. The conformation which is stabilized by this (*S*)-spirolactam was the classical type II′ β-turn, suggesting that a bent conformation may be necessary for NK-1 antagonist activity.

The above two methods have as their formal starting point the natural sequence of the tachykinins. Selective NK-2 antagonists can be obtained from the linear peptide sequence Ac-Leu-Met-Gln-Trp-Phe-Gly-NH_2, which we have reported on previously[7] and whose selectivity has been recently improved upon by Maggi et al.[15] by the replacement of the methionyl with an aspartyl residue.

On the basis of NMR data and energy calculations derived from substance P itself[16,17] and a number of biologically active fragments[18,19] and analogues,[20] it has been postulated that subatance P exists in a bent conformation in the C-terminal portion of the peptide.[21] This conformation is thought to be stabilized by the existence of hydrogen-bonding interactions between the methionyl residue and the backbone or side chain of other residues. In the C-terminal hexapeptide of substance P, [Glp^6]SP_{6-11}, it had been shown that replacement of the Gly^9 residue by derivatives which not only restrict conformation but have the ability to stabilize bends such as L-Pro,[22] D-Pro,[23] and a γ-lactam unit (*R*)-Gly[ANC-2]Leu[20] affect agonist activity at the various receptor subclasses. This implies that the glycyl residue may play a dominant role in establishing the overall conformation of the molecule. We began a synthetic program to determine whether the biologically active conformation at the various subtypes might be mimicked by cyclic peptides. This technique of obtaining peptide antagonists had been successfully employed for other peptide hormones systems including somatostatin,[24] enkephalin,[25] LHRH,[26] and recently CCK.[27] Surprisingly, all previous attempts to achieve this for the tachykinins have been unsuccessful.[28–31] One possible explanation we considered which might account for this was the high conformational mobility of the glycyl residue. It was for this reason that the conformational constraint (*R*)-Gly[ANC-2]Leu, which had been shown to maintain high agonist activity at NK-2 receptors,[20] should be included in these cyclic peptides to provide a conformational lock in this region of the peptide backbone. Later

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(14) The abbreviation (*R*)-Gly[ANC-2]Leu is the shorthand notation which describes the modified dipeptide mimic in 3. This notation signifies the replacement of a hydrogen atom attached to the alpha carbon of the glycyl residue and the hydrogen atom of the amido nitrogen of the leucyl residue by an ethylene (*C*-2) unit in the dipeptide Gly-Leu. The substituted lactam unit so generated has a new chiral center of *R* absolute stereochemistry. The α-carbon of the leucyl residue is unaltered and has the same *S* absolute stereochemistry as before, which by convention for naturally occurring amino acids is assumed to be L unless specified to the contrary.

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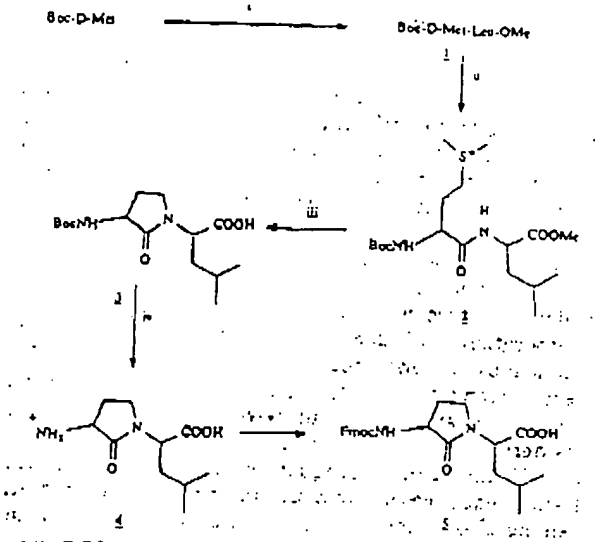
we would be in a position to examine the importance of including this conformational constraint.

Methods

The method for the preparation of the cyclic peptides involved formation of precursor linear peptide hydrazides (Scheme II, part a), conversion to the acyl azides (by the method of Rudinger[32]), and cyclization in a analogous manner to that described by Veber.[24] Preparation of the precursor peptide hydrazides utilized solid-phase techniques similar to those described by Sheppard involving the use of Fmoc amino acids.[33] Polydimethylacrylamide gel resin (Pepsin, CRB) was functionalized by treatment with ethylenediamine and acylated with the Fmoc-protected symmetrical anhydride of an amino acid, which was to act as an internal standard and to facilitate subsequent analyses. Deprotection of the resin by 20% piperidine in DMF (wash cycle A) allowed acylation by the functional handle [4-(hydroxymethyl)benzoic acid[33]]. Subsequent acylation for the assembly of the peptide was by direct analogy with the method of Sheppard involving Fmoc-amino acid symmetrical anhydrides or active esters (glutamine residues). The notable exception to this was the incorporation of the conformationally constrained dipeptide mimic 3, which involved the use of active ester couplings and the use of Boc protection. Preparation of 3 was by analogy with that described previously for the (*S,S*)-epimer[34] using a DCC-mediated coupling of Boc-D-Met with Leu-OMe·HCl. Following this procedure the initial product 1 contained significant quantities of *N*-acylurea (5.3%), which could be removed either by chromatography on silica gel (overall yield, 85.5%) or by recrystallization (58.7%). However, when the coupling procedure was performed with hydroxybenzotriazole present no *N*-acylurea was observed and the product obtained after crystallization (88%) was of sufficient purity to be used directly without further purification. The use of the Boc group to protect 3 rather than Fmoc was solely for convenience. The procedure for the preparation of 3[34] (Scheme I) involved a strong base treatment (NaH) for the lactamization of the S-alkylated dipeptide 2. Fmoc could not be used as N-protection as it is known to be unstable under these conditions. Subsequent exchange of the Boc group in 3 for Fmoc in 5 was performed (Scheme I); however it was deemed unnecessary for this present series of cyclic peptides for two reasons. Firstly, peptides attached to the 4-(hydroxymethyl)benzoyl-functionalized resin 6 are known to be stable to the anhydrous acid conditions necessary to cleave a Boc group,[33] and secondly, the amino acid residues preceding the dipeptide mimic 3 did not have protected functionality in their side chains.

In the cases of the precursor peptides not involving N-terminal glutamine, the final residue was incorporated as either a Boc- or an Fmoc-protected amino acid residue and deprotected under standard conditions before cleavage from the resin by hydrazinolysis (5% hydrazine hydrate in methanol) (Scheme II, part b, procedure A). Exhaustive evaporation to remove excess hydrazine allowed direct

Scheme I [a]



[a] (i) DCC/HOBt/LeuOMe·HCl; (ii) MeI; (iii) NaH; (iv) HCl/dioxane; (v) FMOC-Cl/Na_2CO_3.

conversion of the crude acyl hydrazide to the corresponding acyl azide using isopentyl nitrite.[32] Dilution with precooled DMF (−20 °C) and addition of triethylamine to pH 8–9 liberated the free amine and allowed spontaneous cyclization to occur. In the number of cases involving N-terminal glutamine residues it was anticipated that if the procedure above was adopted a significant degree of pyroglutamyl formation would occur during the hydrazinolysis step. It was for this reason that a modified procedure (Scheme II, part b, procedure B) was adopted in order to minimize this side reaction. In these cases the N-terminal glutaminyl residue was incorporated by the use of Boc-Gln-ONp and the coupling catalyzed by hydroxybenzotriazole (HOBt). The completed peptide was cleaved from the resin by hydrazine with the N-terminal protecting group still present. After removal of the excess hydrazine, the Boc group was removed using anhydrous trifluoroacetic acid or 2 N HCl in dioxane and the acyl azide generated *immediately* at −20 °C. Under these conditions the protonated amino group on the Gln residue should minimize the extent of formation of pyroglutamyl peptides. The lower yields obtained during cyclization of precursor peptides with N-terminal glutamine (Table III) by procedure B possibly indicates that this side reaction has not been totally suppressed. The desired intramolecular reaction to form the homodetic cyclic peptides was however clearly competing favorably with undesired intraresidue cyclization to form pyroglutamyl peptides.

An alternative method which did avoid this side reaction altogether was to alter the position of the bond to be formed during the cyclization step, thus avoiding N-terminal glutaminyl residues. For the peptides which contain the Gly-Leu sequence rather than the dipeptide mimic 3, the obvious choice of C-terminal residue in the linear precursor peptide was the glycyl residue (Scheme III), which not only avoids having Gln as N-terminal residue and hence the possibility of pyroglutamyl formation but also avoids racemization on the cyclization step since the achiral glycyl residue is not susceptible. Where this was not possible, cyclization involving an azide coupling procedure was used to minimize the possibility of racemization at chiral

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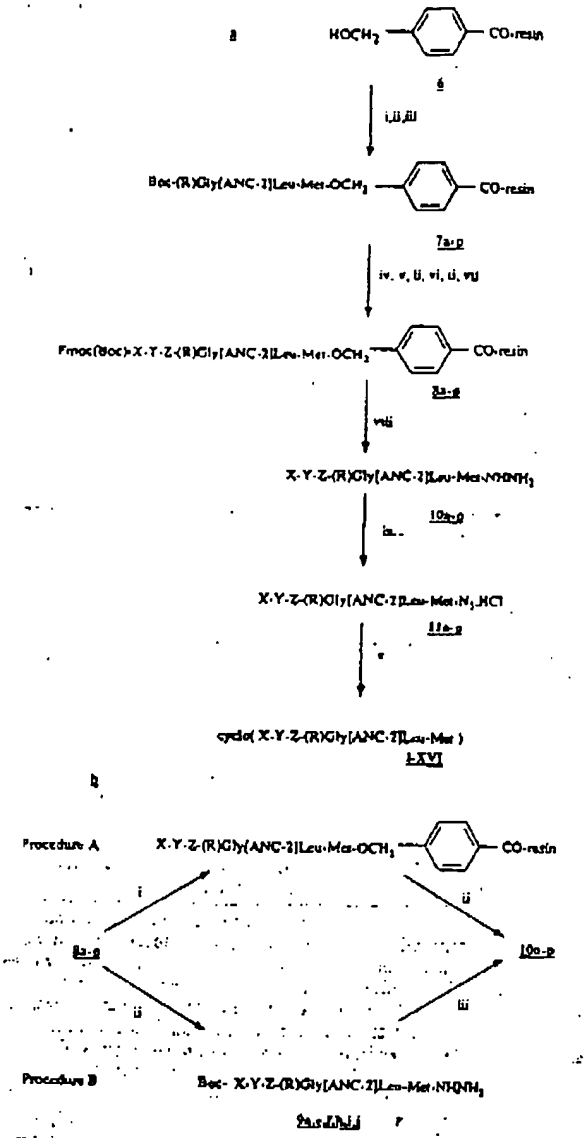
Scheme II



[a] Reagents for part a: (i) (Fmoc-Met)$_2$O/NMM/DMAP; (ii) 20% piperidine/DMF; (iii) 3/DCC/HOBt; (iv) TFA/CH_2Cl_2; (v) (Fmoc-Z)$_2$O; (vi) (Fmoc-Y)$_2$O; (vii) (Fmoc-X)$_2$O; (viii) deprotection and hydrolysis (part b); (ix) HCl/isoamyl nitrite/−20 °C (x) Et_3N. Reagents for part b: (i) 20% piperidine/DMF (for Fmoc removal) or 50% TFA/CH_2Cl_2 (for Boc removal); (ii) 5% hydrazine hydrate/MeOH/16 h; (iii) TFA (30 min) or HCl/dioxane.

C-terminal residues. For cyclic peptides involving the unit 3, alternate choices for the linear precursor peptide sequences were limited for technical reasons. However, when cyclization was attempted using alternative cyclization positions, yields were variable and in one case cyclization failed completely (synthesis of peptide V, method 2).

The purity and identity of the cyclic peptides were characterized (Table IV) by ^1H NMR (360 MHz), amino acid analysis,[35] and FAB-MS. Gel-permeation chromatography, which is a frequently used technique to determine the molecular weight of peptides and proteins,[36] was

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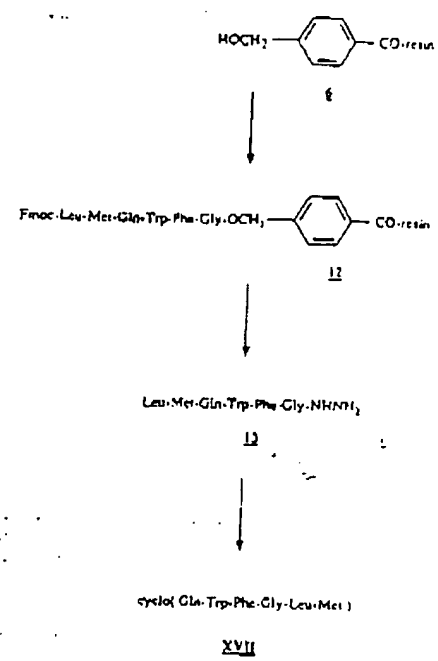
Scheme III



also applied to II, III, IV, VIII, IX, and X using Sephadex G-15 in 50% aqueous acetic acid as solvent. The cyclic peptides were determined to be monomeric species by comparison with the elution time of analogous linear peptides considered to be chemically similar. This solvent was chosen because of the limited water solubility of these cyclic peptides and to minimize absorption effects of the Sephadex by the aromatic residues in the cyclic peptides.[37] The cyclic peptides however consistently eluted later than predicted from their molecular weights, which reflects the lower molecular volume occupied by a cyclic rather than a conformationally less rigid linear peptide. Although this technique was not applied generally, relying on FAB-MS for accurate molecular weight determinations, it was found to be of value in distinguishing between monomeric and oligomeric species.

Discussion

Table I shows the antagonist activity of the cyclic peptides I–XX at the NK-2 receptor (in rat vas deferens against the agonist eledoisin). Five of these peptides act as antagonists with pA_2 values >6. None of the cyclic peptides exhibited any efficacy at NK-1, NK-2, or NK-3 receptors at concentrations up to 10 μM or greater. A number of points can be made relative to structure–activity relationships (SAR) from the results presented in Table I. However, although the cyclization process renders

(36) A number of problems were encountered with the amino acid analyses of both intermediate peptide resin samples and completed cyclic peptides depending on the method of analysis used. Acid hydrolysis of the unit 3 gave two peaks which coeluted with Met and Phe on an ion-exchange-based amino acid analysis system (Waters). The use of OPA as derivatization agent failed to detect secondary amino acids such as (*N*-Me)Phe and Pro whereas the ninhydrin based equivalent system gave interference between Glu and sarcosine (from resin bound peptide analyses). A number of these problems could be largely overcome using Pico-Tag (Waters) precolumn derivatization systems although greater oxidative losses for Met and Trp were found.

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Table I. NK-2 Tachykinin Antagonist Activity of Cyclo(X-Y-Z-D-E)

	X	Y	Z	D	E	apparent pA_2 (RVD)[a] NK-2
I	Gln	Trp	Phe	R[e]	Met	6.7
II	D-Ala	Phe	Phe	R	Met	NA
III	Gly	Phe	Phe	R	Met	NA
IV	Ala	Phe	Phe	R	Met	NA
V	Gln	Phe	Phe	R	Met	4.8
VI	Gln	Npa[b]	Phe	R	Met	5.6
VII	Gly	Npa[b]	Phe	R	Met	5.0
VIII	Gln	D-Trp	Phe	R	Met	4.9
IX	Gln	D-Trp	(*N*-Me)Phe	R	Met	6.4
X	Gln	D-Trp	(*N*-Me)Phe	R	Met_2[c]	5.1
XI	Gly-Gln	Phe	Phe	R	Met	4.6
XII	D-Pro-Gln	Phe	Phe	R	Met	NA
XIII	Aha[d]	Phe	Phe	R	Met	NA
XIV	Gly-Gln	Tyr	Phe	R	Met	5.0
XV	Gly-Gln	D-Trp	Phe	R	Met	5.0
XVI	Gly-Gln	Trp	Phe	R	Met	6.0
XVII	Gln	Trp	Phe	Gly-Leu	Met	8.1
XVIII	Gln	Trp	Phe	D-Pro-Leu	Met	4.7
XIX	Gln	Trp	Phe	Pro-Leu	Met	4.8
XX	Gln	Trp	Phe	Leu	Met	6.2

[a] Tachykinin NK-2 antagonist activity against eledoisin in rat vas deferens. [b] Npa = L-3-(1-naphthyl)alanyl. [c] Cyclic dimer of IX. [d] Aha = 6-aminohexanoyl. [e] R = (*R*)-Gly[ANC-2]Leu (ref 14). NA = not active (apparent $pA_2 < 4.5$).

considerably fewer degrees of freedom when compared with linear peptides, there is still the possibility of conformational mobility and SAR thus derived from large conformationally mobile peptides must be interpreted with this in mind. It does appear however that both ring size and amino acid substitution do play an important role for NK-2 activity. For the seven peptides II–V and XI–XIII which have the native aromatic amino acid sequence Phe-Phe of substance P, it can be seen that weak antagonist activity can be obtained in some cases (for both hexa and heptapeptides). The glutamine residue does appear to be important for activity. Comparison of the peptides containing Gln or Gly at the position immediately prior to the aromatic residues illustrates this. For example peptides III and VII both have lower activity than the corresponding glutaminyl peptides (V and VI, respectively). However, when Gln is present as in peptide V, it is still possible to reduce or abolish activity by introduction of additional residues (Gly or D-Pro) as in XI and XII. One of the most significant substitutions for increasing NK-2 antagonist activity in hexapeptide V was replacement of Phe by L-Trp (peptide I, $pA_2 = 6.7$). In linear peptide antagonists, subetitution of residues by D-Trp commonly increases antagonist activity. This increase in potency at NK-2 was not observed in these cyclic peptides. Substitution of L-Trp (peptide I, $pA_2 = 6.7$) by D-Trp (peptide VIII, $pA_2 = 4.9$) results in significant loss of activity. However when substitution was by D-Trp in combination with *N*-methylphenylalanine (peptide IX), both potency and selectivity (Table II) were affected. In particular, activity at NK-1 was increased significantly ($pA_2 = 6.6$, Table II). For the heptapeptides containing X = Gly-Gln, sensitivity to aromatic subetitution was similar, as can be seen by comparison of peptides XI, XVI, XIV, and XV. Again, L-Trp appears to be preferred to the other aromatic residues L-Phe, L-Tyr, and D-Trp.

The reasons for the inclusion of the conformational constraint (*R*)-Gly[ANC-2]Leu in the initial series of cyclic peptides, as described earlier, was intended to provide conformational rigidity in this conformationally mobile portion of the cyclic peptide backbone. Introduction of this lactam unit in the C-terminal hexapeptide sequence of substance P is compatible with high NK-2 agonist activity. However, the conformation which this imposes is clearly not optimal for activity at NK-2 receptors as a 20-fold increase in activity is observed in I when it is replaced by the native Gly-Leu sequence in XVII. The cyclic peptide antagonists are not congeneric with the linear peptide agonists and the SAR is clearly different for these two series. Introduction of the lactam unit into the native Gly-Leu sequence of $[Glp^6]SP_{6-11}$ also results in loss of agonist affinity of some 4-fold.[20] However a more dramatic difference is observed in these two series of compounds when one examines the effect of introduction of D-Pro in place of Gly. As an agonist, the activity of $[Glp^6,Pro^9]SP_{6-11}$ at NK-2 is comparable with that of eledoisin.[38] In the cyclic peptide XVII, replacement of the Gly residue with D-Pro almost totally abolishes activity.

Table II. Selectivity for Different Tachykinin Receptor Types[39]

cyclic peptide	pA_2 values[a] NK-1 (GPI)	NK-2 (RVD)	NK-3 (RPV)
I	NA	6.7 (CL = 6.1, 7.2) slope 1.0	4.9*
IX	6.6	6.4 (±0.3, $n = 3$)	5.7*
X	7.0	5.1*	5.6*
XIV	NA	5.0*	5.4*
XVII	5.7 (±0.1, $n = 4$)	8.1 (CL = 7.6, 8.4) slope = 0.83	5.1 (±0.2, $n = 4$)

[a] pA_2 values were obtained by the line of best fit (least squares) to the Schild regression (ref 41) using three or more concentrations of antagonist with eledoisin as agonist, where the values in parentheses denote the 95% confidence limits (CL). Otherwise the pA_2 values were the mean of three or four observations obtained from the standard equation for the Schild regression analysis, assuming unit slope, where the values in parentheses denote the standard error of the mean (±). The values marked with * were obtained from a single concentration of antagonist from the standard equation for the Schild regression analysis, assuming unit slope. GPI = guinea pig ileum. RVD = rat vas deferens. RPV = rat portal vein. NA = not active; $pA_2 < 5.0$.

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Table III. Synthesis and Characterization of Peptide Intermediates

cyclic peptide	8–10 terminal intermediate 8	internal residue amino acid	amino acid analysis[a] reference 8	cleavage procedure	HPLC,[b] t_R (min) 9	HPLC,[b] t_R (min) 10	yield[c] %
I	a Boc-Gln	Ala	Glu, 0.91; Phe, 0.92; Ala, 1.0	B	16.2	13.2	33
II	b Boc-D-Ala	Gly	Ala, 0.89; Phe, 1.92; Gly, 1.0	B	19.2	14.2	35
III	c Fmoc-Gly	Gly	Phe, 2.06; Gly, 1.83	A		13.4	59
IV	d Fmoc-Ala	Gly	Ala, 0.94; Phe, 2.06; Gly, 0.89	A		13.8	50
V	e Boc-Gln	Ala	Phe, 2.11; Ala, 1.0	B	16.2	12.3	26
VI	f Boc-Gln	Leu	ND	B	17.8	14.2	23
VII	g Fmoc-Gly	Leu	ND	A		14.7	33
VIII	h Boc-Gln	Ala	ND	B	16.2	13.1	27
IX	i Boc-Gln	Leu	ND	B	18.1	15.7	12
X	j Boc-Gln	Leu	ND	B	18.1	15.7	11
XI	k Fmoc-Gly	Ala	Gly, 0.99; Glu, 1.03; Phe, 2.01; Ala, 1.00	A		16.1	38
XII	l Boc-(*R*)-Gly[ANC-2]Leu	Ala	Pro, 1.04; Glu, 0.93; Phe, 1.97; Ala, 1.00	A		12.6[d]	49
XIII	m Boc-Aha	Ala	Phe, 1.73; Ala, 1.00	A		12.6	43
XIV	n Boc-Gly	Leu	ND	A		11.5	22
XV	o Fmoc-Gly	Ala	Gly, 1.02; Phe, 0.89; Met, 1.10; Ala, 1.0	A		14.0	9.2
XVI	p Fmoc-Gly	Ala	Gly, 0.97; Phe, 0.90; Met, 0.98; Ala, 1.0	A		13.0	40
XVII	q Boc-Gln	Ala	ND	B	16.5	13.0	ND
XVIII	r Boc-Gln	Ala	ND	B	17.8	15.0	5.8
XIX	s Boc-Gln	Ala	ND	B	16.8	13.2	ND
XX	t Fmoc-Leu	Ala	ND	A		13.7	ND

[a] See ref 35. [b] μ-Bondapak C-18, 5–95% CH_3CN linear gradient over 30 min at 2 mL/min (0.1% TFA in aqueous solution) ND = not determined. [c] Overall yield based on derivatized starting resin. [d] Retention time of acyl hydrazide (sequence not as in 10).

Table IV. Characterization of Cyclic Peptides I–XX

cyclic peptide	formula	FAB-MS $(M + H)^{a,b}$ (m/e)	HPLC,[c] t_R (min)	% purity	TLC (R_f)[d] A	B	C	D	amino acid analyses[d,e]
I	$C_{40}H_{52}N_8O_7S$	790, 788[b]	16.88	99.2	0.27	0.49	0.23	0.86	(B) Glu, 1.00; Phe, 1.03; X,[e] 0.96; Met, 0.97
II	$C_{39}H_{48}N_8O_8S$	693, 715[a]	19.39	>99.5	0.69	0.79	0.64	0.92	(A) Ala, 0.95; Phe, 2.05; Met, 2.12
III	$C_{38}H_{46}N_8O_8S$	679, 677[b]	18.45	95.7	0.54	0.78	0.40	0.91	(A) Gly, 0.95; Phe, 2.06
IV	$C_{39}H_{48}N_8O_8S$	693, 691[b]	18.92	>99.5	0.52	0.78	0.38	0.92	(A) Ala, 0.96; Phe, 2.04
V	$C_{39}H_{51}N_7O_7S$	750, 748[b]	16.87	>99.5	0.38	0.68	0.44	0.92	(B) Glu, 0.98; Phe, 2.05, X,[e] 1.08; Met, 0.97
VI	$C_{42}H_{53}N_7O_7S$	800, 728[b]	18.51	97.2	0.40	0.56	0.52	0.95	(B) Glu, 1.08; Npa,[f] 0.77; Phe, 1.03; X,[e] 1.16; Met, 0.96
VII	$C_{38}H_{48}N_8O_8S$	729	18.51	97.8	0.54	0.57	0.32	0.89	(B) Gly, 0.97; Npa,[f] 1.05; Phe, 0.96; X,[e] 1.02
VIII	$C_{40}H_{52}N_8O_7S$	789, 787[b]	20.22	99.2	0.47	0.79	0.48	0.95	(B) Glu, 0.96; Met, 1.04; Phe, 0.99
IX	$C_{41}H_{54}N_8O_7S$	825,[a] 801[b]	17.93	95.1	0.22	0.53	0.22	0.88	(B) Glu, 1.04; Met, 0.96
X	$C_{82}H_{108}N_{16}O_{14}S_2$	1627[a]	22.35	90.1	0.16	0.41	0.19	0.86	(B) Glu, 1.99; Met, 2.01
XI	$C_{40}H_{54}N_8O_8S$	807, 805[b]	16.53	95.9	0.16	0.35	0.11	0.86	(A) Gly, 0.93; Glu, 1.00; Phe, 2.07
XII	$C_{43}H_{58}N_8O_8S$	847, 845[b]	17.62	>99.5	0.14	0.34	0.11	0.81	(A) Pro, 1.01; Glu, 0.99; Phe, 2.00
XIII	$C_{44}H_{62}N_8O_8S$	863, 861[b]	17.20	>99.5	0.29	0.56	0.22	0.88	(A) Glu, 0.97; Phe, 2.12; Aha,[g] 0.91
XIV	$C_{40}H_{54}N_8O_9S$	823, 821[b]	15.41	>99.5	0.06	0.13	0.05	0.82	(A) Gly, 1.05; Glu, 0.99; Tyr, 0.97; Phe, 0.99
XV	$C_{42}H_{56}N_9O_8S$	846,[a] 844[b]	16.87	94.1	0.06	0.13	0.07	0.84	(B) Glu, 1.01; Gly, 1.01; Phe, 1.03; Met, 0.95
XVI	$C_{42}H_{56}N_9O_8S$	844[b]	16.73	97.5	0.12	0.29	0.10	0.83	(B) Gly, 1.04; Glu, 0.96; Phe, 1.01; X,[e] 0.97; Met, 1.09
XVII	$C_{38}H_{50}N_8O_7S$	763, 761[b]	16.85	>99.5	0.15	0.34	0.11	0.88	(B) Glu, 0.96; Phe, 1.02; Gly, 1.03; Leu, 0.99
XVIII	$C_{41}H_{54}N_8O_7S$	803, 801[b]	18.13	99.0	0.14	0.30	0.10	0.87	(B) Glu, 1.05; Phe, 0.92; Pro, 1.13; Leu, 0.97
XIX	$C_{41}H_{54}N_8O_7S$	803, 801[b]	17.18	98.6	0.21	0.51	0.17	0.91	(A) Glu, 1.01; Pro, 1.08; Met, 0.93; Leu, 1.01; Phe, 0.96
XX	$C_{38}H_{47}N_7O_6S$	706	18.13	97.0	0.08	0.11	0.18	0.84	(A) Glu, 1.03; Met, 1.03; Leu, 0.96; Phe, 0.98; Trp, 0.94

[a] FAB-MS (M + Na). [b] FAB-MS (M – H). [c] Analytical HPLC was performed on a Waters μ-Bondapak C18 column using a linear gradient of 5–95% acetonitrile using water containing 0.1% TFA, v/v. Purity estimates were by UV determination O.D. 254 nm using a Shimatzu CR5A integrator. [d] Amino acid analyses were performed on hydrolyzates (6 N HCl/18 h/110 °C) on a Waters amino acid analyzer (A) using ion exchange and development with ninhydrin or (B) Waters "Pico-Tag" system using separation on RP18 columns of precolumn derivatized (PITC) amino acids (ref 35). [e] X refers to analysis of the hydrolyzed lactam unit (*R*)-Gly[ANC-2]Leu (ref 14) under conditions B above. [f] Npa = L-3-(1-naphthyl)alanine. [g] Aha = 6-aminohexanoic acid. [h] See text for definition of the solvent systems.

Table II shows the selectivity data for some of these cyclic peptides. It can be seen that peptides I and XVII show good selectivity for the NK-2 receptor compared with NK-1 and NK-3. However, activity is not restricted to NK-2 in these cyclic peptides. Substitution of the L-*N*-methylphenylalanine residue into VIII gave IX with increased antagonist activity at NK-1 and NK-3 receptor subtypes. However during the cyclization of this peptide the dodecapeptide X was also isolated and demonstrated selectivity for NK-1 receptors in guinea pig ileum (GPI, pA_2 = 7.0). Therefore not only can cyclic peptides give potent NK-2 antagonists, but peptides IX and X illustrate the possibility of obtaining antagonists which are potent at the other NK-1 and NK-3 sites.

Conclusion

Cyclic peptides with tachykinin antagonist activity have been identified with amino acid residues similar to the native peptide sequences. NK-2 and NK-1 selectivity has been shown in peptides XVII and X, respectively. Peptide XVII is one of the most potent NK-2 selective antagonists reported to date (pA_2 = 8.1). The peptides presented here illustrate the scope for activity where the range of substitution is not exhaustive by any means. There is a real prospect that improved activity might be achieved in these peptides by optimization of the amino acid residues. These antagonists, together with the NK-1-selective antagonists of Ward,[13] will provide a means of probing the

(39) McKnight, A. T.; Maguire, J. J. Comparison of the receptors for tachykinins in the vas deferens and the portal vein of the rat. *Br. J. Pharm.* 1987, *90*, 103P.

conformational requirements of the binding domains in or near the NK-2 and NK-1 receptors for the tachykinins. The cyclic peptides in particular, by virtue of the lack of conformational mobility, are amenable to conformational analysis by NMR and X-ray techniques.

Experimental Section

Abbreviations used follow IUPAC–IUB nomenclature.[40] Additional abbreviations are (*R*)-Gly[ANC-2]Leu,[14] (2*S*)-2-[(3*R*)-3-amino-2-oxopyrrolidin-1-yl]-4-methylpentanoyl; Aha, 6-aminohexanoic acid; Npa, L-3-(1-naphthyl)alanine; TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; t_R, retention time (min) in a standard 30-min gradient; RP-18, reverse-phase octadecylsilyl; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; DMAP, 4-(dimethylamino)pyridine; NMM, *N*-methylmorpholine; TEA, triethylamine.

Melting points were determined on a Büchi melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AM-360 and AM-250 (360 and 250 MHz) spectrometers and expressed as parts per million from Me_4Si as standard. HPLC analysis was performed on a Waters gradient system equipped with variable-wavelength detector and a Shimatzu integrator. A μ-Bondapak C-18 (4.66 i.d. × 25 cm) column was used. Compounds were detected at 254 and at 234 nm. TLC was performed on precoated silica gel plates: Merck/UV 254. The following solvent systems were used: (A) $CHCl_3$/MeOH (9:1), (B) $CHCl_3$/MeOH/AcOH (85:10:5), (C) $CHCl_3$/MeOH/H_2O (90:10:1), (D) *n*-BuOH/EtOAc/AcOH/H_2O (1:1:1:1). Peptides were visualized by ninhydrin spray and Cl_2/tolidine/KI.

N-(*tert*-Butoxycarbonyl)-D-methionyl-L-leucine Methyl Ester (1). To a solution of Boc-D-Met (2.0 g, 8.02 mmol) and HOBt (1.22 g, 8.00 mmol) in DMF (10 mL) and CH_2Cl_2 (20 mL), cooled to 0 °C was added a solution of DCC (1.65 g, 8.0 mmol) in CH_2Cl_2 (5 mL). After the solution had been stirred at 0 °C for 30 min, LeuOMe·HCl (1.46 g, 8.04 mmol) and NMM (0.88 mL, 3.02 mmol) were added, and the solution was stirred at 0 °C for a further 30 min and then at 5 °C for 16 h. The solution was filtered, and the filtrate together with CH_2Cl_2 washings were evaporated to dryness. The residue was dissolved in ethyl acetate (50 mL) and was washed successively with 10% aqueous sodium carbonate (40 mL, 25 mL), water, 10% aqueous citric acid (25 mL, 25 mL), water, and saturated brine. All aqueous washings were back-extracted with ethyl acetate and the combined ethyl acetate phases were dried over Na_2SO_4 and evaporated to an oil. Diethyl ether (40 mL) was added to dissolve the residue. The solution was filtered to remove remaining DCU and on evaporation to a small volume the product *N*-(*tert*-butoxycarbonyl)-D-methionyl-L-leucine methyl ester (1) crystallized (2.67 g, 88%): mp 57.5–60 °C; $[\alpha]^{25}_D$ −5.15° (*c* = 1, MeOH); ^{1}H NMR (250 MHz, d^6-acetone) δ 7.54 (1 H, br d, CHCONH), 6.2 (1 H, br d, OCONH), 4.5 (1 H, m, NHCH), 4.2 (1 H, m, =NCH), 3.65 (3 H, s, OMe), 2.45 (2 H, t, SCH_2), 2.05 (3 H, s, SMe), 2.1–1.5 (5 H, m, CH_2CH_2S + $CHCH_2CH(CH_3)_2$), 1.4 (9 H, s, $(CH_3)_3$), 0.9 (6 H, dd, $(CH_3)_2CH$).

(2*S*)-2-[(3*R*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxopyrrolidin-1-yl]-4-methylpentanoic Acid (3). Compound 1 (45.94 g, 0.122 mol) was stirred with methyl iodide (220 mL) for 3 days. The methyl iodide was removed by repeated evaporation and dissolution in CH_2Cl_2 (three times) to give a foam. This crude *S*-methylsulfonium iodide (2) was dissolved in DMF/CH_2Cl_2 (240 mL; 1:1) at 0 °C under an atmosphere of N_2 and sodium hydride (10.65 g, 55–60% dispersion in oil, 0.244 mol) added. After stirring of the solution at 0 °C for 2 h, methyl acetate (375 mL) and ethyl acetate (435 mL) were added, followed by water (11 mL). The mixture was stirred at ambient temperature for 16 h under an atmosphere of nitrogen. The product was extracted into water (2 × 1 L) and 1% sodium carbonate (1 L). The aqueous phases were acidified by addition of solid citric acid to pH 4, and the product was extracted into CH_2Cl_2 (three times). The organic phases were washed separately with water and saturated brine and then combined and dried over Na_2SO_4. Removal of the solvent in vacuo gave a viscous oil which crystallized on standing with ethyl acetate/hexane (1:1) to give crude 3 (15.75 g). Recrystallization from hot ethyl acetate/hexane and cooling to 5 °C gave pure 3 (10.25 g, 27%): mp 163–168 °C dec; $[\alpha]^{25}_D$ +21.3° (*c* = 1, MeOH); ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.7 (1 H, br s, NH), 4.77 (1 H, t, *J* = 8.3 Hz, CHCOOH), 4.22 (1 H, br s, CHNH), 3.44 (1 H, dt, CH_AH_BN), 3.2 (1 H, t, *J* = 8.9 Hz, CH_AH_BN), 2.5 (1 H, m, $CH_AH_BCH_2N$), 1.8 (1 H, m, $CH_AH_BCH_2N$), 1.7 (2 H, t, *J* = 7.9 Hz, $CHCH_2CH$), 1.49 (1 H, m, $CH(CH_3)_2$), 1.47 (9 H, s, $(CH_3)_3C$), 0.94 (6 H, dd, *J* = 6.65 Hz, $(CH_3)_2CH$). Anal. ($C_{15}H_{26}N_2O_3$) C, H, N.

(2*S*)-2-[(3*R*)-3-[[(9-Fluorenylmethoxy)carbonyl]amino]-2-oxopyrrolidin-1-yl]-4-methylpentanoic Acid (5). Compound 3 (0.52 g, 1.65 mmol) was treated twice with 2 M HCl/dioxane (5 mL) (1 h, 3.5 h) until no starting material was visible by TLC (B). The solvent was evaporated in vacuo and residual solid washed with ether to give (2*S*)-2-[(3*R*)-3-amino-2-oxopyrrolidin-1-yl]-4-methylpentanoic acid hydrochloride (4), 0.41 g (99%), mp 207–209 °C. This hydrochloride salt (0.34 g, 1.36 mmol) was dissolved in 10% aqueous Na_2CO_3 (5 mL) and the solution cooled in an ice bath. A solution of 9-fluorenyl chloroformate (0.39 g, 1.51 mmol) dissolved in dioxane (2.5 mL) was added, and after stirring of the solution for 2.5 h at room temperature it was poured into water (75 mL) and washed with diethyl ether (2 × 3 mL). The aqueous phase was acidified by addition of concentrated hydrochloric acid until pH 1–2 and the product was extracted with ethyl acetate (2 × 50 mL). The organic phases were washed separately with water, combined, and dried over sodium sulfate. Evaporation of the solvent gave an oil which crystallized on standing. The solid was washed with hexane and dried to give 5 (0.32 g, 53%): mp 130 °C; $[\alpha]^{25}_D$ +18° (*c* = 0.2, MeOH); ^{1}H NMR (360 MHz, $CDCl_3$) δ 7.74 (2 H, d, *J* = 7.5 Hz, aryl), 7.58 (2 H, d, *J* = 7.4 Hz, aryl), 7.39 (2 H, t, *J* = 7.3 Hz, aryl), 7.30 (2 H, t, *J* = 7.5 Hz, aryl), 6.66 (1 H, v br s), 5.67 (1 H, d, *J* = 5.4 Hz), 4.85 (1 H, br t), 4.38 (3 H, m), 4.21 (1 H, t, *J* = 6.95 Hz), 3.7 (1 H, br s), 3.48 (1 H, br t), 2.66 (1 H, br s), 1.89 (1 H, br t), 1.77 (2 H, t, *J* = 6.9 Hz), 1.49 (1 H, br m), 0.94 (6 H, t, *J* = 7.15 Hz, $(CH_3)_2CH$). Anal. ($C_{25}H_{28}N_2O_5 \cdot 0.25H_2O$) C, H, N.

Cyclo(Gln-Trp-Phe-(*R*)-Gly[ANC-2]Leu-Met) (I). Procedure B. (a) Functionalized Resin 4-HOCH$_2$C$_6$H$_4$CO-Ala-resin (HOMB-Ala-resin) (6). Polydimethylacrylamide resin (Cambridge Research Biochemicals; 2.96 g, 2.96 mmol of functional sarcosine methyl ester) was treated with diaminoethane for 16 h. The resin was washed on a Vega TM 250[e] solid-phase peptide synthesizer with wash cycle A (DMF, 10 × 1 min; 10% diisopropylethylamine in DMF, 3 × 1 min; DMF, 10 × 1 min). To the resin was added the preformed symmetrical anhydride of Fmoc-L-Ala (8.9 mmol) dissolved in DMF (20 mL) for 40 min. The resin was washed with wash cycle B (DMF, 10 × 1 min; 20% piperidine in DMF, 3 min + 7 min; DMF 10 × 1 min) followed by addition of the preformed symmetrical anhydride of 4-(hydroxymethyl)benzoic acid (8.9 mmol) dissolved in DMF (20 mL) for 40 min and finally wash cycle A.

(b) **Intermediate Resin Boc-(*R*)-Gly[ANC-2]LeuMet-OMB-resin (7).** The resin (6) was acylated with the preformed symmetrical anhydride of Fmoc-Met (8.9 mmol) in the presence of NMM (8.9 mmol) and a catalytic amount of DMAP (0.89 mmol) in DMF (20 mL) for 20 min, followed by deprotection with wash cycle B and acylation with (2*S*)-2-[(3*R*)-3-[(butoxycarbonyl)-amino]-2-oxopyrrolidin-1-yl]-4-methylpentanoic acid [Boc-(*R*)-Gly[ANC-2]LeuOH[14] (3) (5.9 mmol)] [previously preactivated with DCC (5.9 mmol) and HOBt (5.9 mmol) for 30 min at 0 °C in CH_2Cl_2/DMF (2:1)] in DMF (25 mL) for 16 h. Excess reagents were then removed using wash cycle A followed by washing with CH_2Cl_2.

(c) **Boc-Gln-Trp-Phe-(*R*)-Gly[ANC-2]Leu-Met-OMB-resin (8a).** The *tert*-butoxycarbonyl group was removed from 7 by treatment of the resin with TFA/CH_2Cl_2 (1:1) containing 1% ethanedithiol (5 and 25 min). The resin was then washed with CH_2Cl_2 followed by wash cycle A and coupled with the preformed symmetrical anhydride of Fmoc-Phe (8.9 mmol) for 1.25 h. After washing with DMF (5 × 1 min) the resin was divided into two separate portions. One portion (2.4 mmol) was deprotected using wash cycle B followed by acylation with the symmetrical anhydride of Fmoc-Trp (7.08 mmol) for 45 min in DMF (15 mL).

(40) Nomenclature and symbolism for amino acids and peptides. *Eur. J. Biochem.* 1984, *138*, 9–37.

The resin was washed with wash cycle B, acylated with Boc-Gln-ONp (7.10 mmol) in DMF (30 mL) in the presence of HOBt (7.14 mmol) for 120 min, followed by washing with wash cycle A. Finally the resin was washed successively with CH_2Cl_2 and diethyl ether before being dried under the vacuum of an oil pump to give protected resin 8a (4.38 g). Amino acid analysis: (see Table III).

(d) **Boc-Gln-Trp-Phe-(*R*)-Gly[ANC-2]Leu-Met-NHNH$_2$ (9a) (Procedure B).** Resin 8a was treated with 5% hydrazine hydrate in methanol (70 mL) for 16 h. The solution was filtered and the filtrate evaporated to dryness followed by desiccation of the residue under the vacuum of an oil pump over concentrated sulfuric acid (24 h). The protected peptide hydrazide 9a was obtained, 1.71 g, estimated purity = 95% (HPLC, t_R = 16.2 min).

(e) **Gln-Trp-Phe-(*R*)-Gly[ANC-2]Leu-Met-N$_3$ Hydrochloride (11a).** The protected peptide hydrazide 9a (1.69 g, 1.83 mmol) was treated with anhydrous TFA (100 mL) for 30 min. Evaporation gave a foam which was dissolved in DMF (40 mL) and cooled to −25 °C under an atmosphere of nitrogen. HCl (12.7 M) in THF (0.72 mL) was added followed by isopentyl nitrite (0.37 mL, 2.75 mmol) and the resultant solution stirred at −25 °C for 2 h to give the peptide acyl azide 11a (HPLC, t_R = 13.0 min).

(f) **Cyclo(Gln-Trp-Phe-(*R*)-Gly[ANC-2]Leu-Met (I).** The solution of peptide acyl azide 11a was diluted with precooled (−25 °C) DMF (3000 mL). Triethylamine was added dropwise until pH 8.5 (ca. 10 mL) and the solution was left stirring at −25 °C for 20 h. The solvent was removed by evaporation in vacuo and the residue, dissolved in $CHCl_3$ (175 mL), was washed with water (2 × 175 mL). Each aqueous wash was back-extracted with fresh $CHCl_3$ (50 mL). The combined $CHCl_3$ phases were dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel (E. Merck, Lobar, Lichroprep, size C) eluting the product with a linear gradient from $CHCl_3$ (500 mL) to $CHCl_3$/MeOH/AcOH (85:10:5, 500 mL) at 5.4 mL/min, with 2-min fraction collection. The fractions containing product (46–51) were combined, evaporated, and chromatographed in three separate portions by reverse-phase chromatography (E. Merck, Lobar, Lichroprep, RP-8, size C). The product was eluted with a linear gradient from H_2O/CH_3CN/TFA (90:10:0.1, 1000 mL) to H_2O/CH_3CN/TFA (10:90:0.1, 1000 mL) at 5.4 mL/min, with 2-min fraction collection. The fractions which contained pure material (81–86 incl) were combined and freeze-dried to give I, 678 mg (see Table IV for analytical data of final peptides).

Preparation of Cyclo(D-Ala-Phe-Phe-(*R*)-Gly[ANC-2]-Leu-Met) (II). (a) **Boc-D-Ala-Phe-Phe-(*R*)-Gly[ANC-2]Leu-Met-OMB-Gly-resin (8b).** Stock resin Boc-(*R*)-Gly[ANC-2]Leu-OMB-Gly-resin (0.63 g, peptide loading = 0.56 mmol/g determined by amino acid analysis, 0.35 mmol) prepared in an analogous manner to that described for 7 was deprotected by treatment with 50% TFA in CH_2Cl_2 (containing 1% ethanedithiol, 5 + 25 min) and the resin was neutralized (wash cycle A). Successive acylation and deprotection cycles with $(FmocPhe)_2O$ (2.08 mmol, 1.75 h), $(FmocPhe)_2O$ (2.08 mmol, 2.25 h), and $(Boc\text{-}D\text{-}Ala)_2O$ (2.08 mmol, 1 h) gave 8b, 0.71 g (0.32 mmol, Gly).

(b) **Boc-D-Ala-Phe-Phe-(*R*)-Gly[ANC-2]Leu-Met-NHNH$_2$ (9b).** A portion of Boc-protected resin 8b (0.58 g, 0.26 mmol of Gly, 0.22 mmol of Ala) was treated with 5% hydrazine hydrate in methanol (16 h). The residual resin was removed by filtration, which after washing and hydrolysis indicated that >98% of the peptide had been cleaved. The filtrate and methanol washings were evaporated in vacuo to remove remaining hydrazine to give crude Boc-protected hexapeptide acyl hydrazide 9b (0.23 mmol): TLC (B) R_f (CMA) 0.53; HPLC (t_R = 19 min); estimated purity = 95%; amino acid analysis, Ala, 1.02; Phe, 1.98; Met, 1.38.[36] An analytical sample was partially oxidized by treatment with H_2O_2 in acetic acid which gave peaks on HPLC at 17.3 and 19.2 min, indicating that the unpurified sample of 9b above did not contain any methionine sulfoxide.

(c) **Cyclo(D-Ala-Phe-Phe-(*R*)-Gly[ANC-2]Leu-Met (II).** Crude 9b (0.32 mmol) was treated with 2.2 M HCl/dioxane (2 × 30 min) and evaporated to dryness (HPLC, 14.8 min). The residual hydrochloride salt (242 mg, 0.30 mmol) was dissolved in DMF (6.4 mL), and 4.2 M HCl/THF (1.5 mmol) was added and the cooled solution (−25 °C) stirred under an atmosphere of nitrogen while isopentyl nitrite (0.06 mL, 0.45 mmol) was added. After stirring at −25 °C for 4 h the solution was diluted by addition of DMF (500 mL, 0.32 mmol, precooled to −25 °C) followed by TEA (2.2 mL) to pH 8 and the solution kept at −30 °C for 64 h. The solvent was removed in vacuo at 30–35 °C and the residue chromatographed on silica gel (Merck, Lobar, size C, Si60) at 5 mL/min eluting with a gradient from $CHCl_3$ (500 mL) to $CHCl_3$/MeOH/AcOH (85:10:5, 500 mL) to give the purified II (95.5 mg, 43.1%): HPLC, t_R = 20.5 min; TLC (B) R_f (CMA) 0.63, single spot. The cyclic peptide was shown to be monomeric by chromatography on Sephadex G-15 (estimated molecular weight = 575) and FAB-mass spectrometry. Anal. ($C_{36}H_{48}N_6O_5 \cdot 0.5H_2O$) C, H, N.

Cyclo(Gln-Phe-Phe-(*R*)-Gly[ANC-2]Leu-Met) (V). Method 1. Procedure B. Peptide V was prepared in an analogous manner to that described for I (Scheme II, procedure B): amino acid analysis, Glu, 0.98; Phe, 2.05; Met, 0.97; FAB-MS negative ion *m/e* 748 (M − H), positive ion *m/e* 750 (M + H).

Method 2. Attempted Alternate Ring-Forming Strategy. H-(*R*)-Gly[ANC-2]Leu-Met-Gln-Phe-Phe-OMB-Ala-resin. Resin 6 (0.98 g, 0.89 mmol) was acylated successively with $(FmocPhe)_2O$/NMM/DMAP (1:1:0.1) (5.2 mmol, 20 min), $(FmocPhe)_2O$ (5.2 mmol, 45 min), and Fmoc-GlnONP/HOBt (1:1) (2.85 mmol, 1.5 h) to give the protected resin-bound tripeptide (1.37 g). A portion of this resin (0.68 g) was acylated with $(FmocMet)_2O$ (2.6 mmol, 65 min) and Boc(*R*)-Gly[ANC-2]-LeuOH/HOBt/DCC (1:1:1) (0.88 mmol, 60 min) and finally deprotected using 50% TFA/CH_2Cl_2 (containing 1% ethanedithiol, 5 + 25 min) washed with CH_2Cl_2 and diethyl ether and dried in vacuo to give 0.87 g of the dried resin: amino acid analysis, Glu, 1.10; Ala, 1.00 (0.42 mmol); Phe, 2.05.

Attempted Cyclization To Give V. The dried resin above was treated with 5% hydrazine hydrate in methanol (16 h) at room temperature and then the solution was filtered. The residual resin was washed with DMF (50 mL), and the combined filtrates were evaporated to give (*R*)-Gly[ANC-2]Leu-Met-Gln-Phe-Phe-NHNH$_2$ (475 mg; HPLC, t_R = 12.8 min, estimated purity > 95%). The hydrazide dissolved in DMF (12.8 mL) was cooled to −25 °C under an atmosphere of nitrogen, followed by addition of 7.2 M HCl/THF (0.42 mL, 3 mmol) and isopentyl nitrite (0.122 mL, 0.91 mmol). After 3.5 h at −25 °C HPLC indicated that formation of the acyl azide (16.3 min) was almost complete. The solution was diluted with precooled DMF (600 mL) and TEA (1.3 mL) added until pH 8.5. After 3.5 days the solvent was removed in vacuo; however no material corresponding to cyclic peptide had formed (as indicated by both TLC and HPLC).

Cyclo(Gln-Npa-Phe-(*R*)-Gly[ANC-2]Leu-Met) (VI). The *N*-*tert*-butoxycarbonyl-protected peptide hydrazide 9f was prepared in a manner similar to that described for 9a. Fmoc-Met, Boc-(*R*)-Gly[ANC-2]LeuOH, and Fmoc-Phe were coupled onto the solid-phase resin under conditions identical with those described for I. At the appropriate stage in the solid-phase synthesis was coupled *N*-[(9-fluorenylmethoxy)carbonyl]-L-2-(1-naphthyl)alanine [Fmoc-Npa, prepared from commercially available L-3-(1-naphthyl)alanine (Chemalog) by treatment with fluorenylmethyl chloroformate in aqueous sodium carbonate, mp 181–183 °C, $[\alpha]^{23.5}_D$ −78.6° (*c* = 0.87, DMF)] as the preformed symmetrical anhydride for 1.25 h. Boc-Gln-ONP was finally coupled to the resin after prior removal of the Fmoc group (wash cycle B). The crude peptide hydrazide (9f) was obtained by treatment of the resin with 5% hydrazine hydrate in methanol for 16 h followed by filtration and evaporation of the filtrate and exhaustive drying in vacuo. Removal of the *N*-*tert*-butoxycarbonyl group from the peptide hydrazide was performed with 2.3 M HCl/dioxane followed by cyclization under the standard conditions described earlier. Purification of the crude cyclic peptide was performed using silica gel chromatography (E. Merck, Lobar, size C, column gradient from chloroform (1000 mL) to chloroform/methanol/acetic acid (85:10:5; 1000 mL) followed by recrystallization from ethyl acetate/hexane to give VI with an estimated purity = 97.2% (HPLC).

Cyclo(Gln-D-Trp-(*N*-Me)Phe-(*R*)-Gly[ANC-2]Leu-Met) (IX) and Cyclo(Gln-D-Trp-(*N*-Me)Phe-(*R*)-Gly[ANC-2]Leu-Met)$_2$ (X). Resin 7 was deprotected and coupled sequentially with Fmoc-(*N*-Me)Phe (1.5 h) and Fmoc-D-Trp (22.5 h) as their corresponding symmetrical anhydride and Boc-Gln-ONp (2 h)

coupled in the presence of HOBt. After washing, the protected peptide was removed from the resin by treatment with 5% methanolic hydrazine hydrate (procedure B). The protected peptide hydrazide 9i obtained after evaporation of the filtrate was deprotected and cyclized as described. Analytical HPLC (μ-Bondapak) indicated the presence of two major products. These were separated by silica gel chromatography and crystallization. The earlier eluting product on analytical HPLC (μ-Bondpak), IX, was characterized as the monomeric cyclic peptide by both mass spectrometry [FAB, positive ion m/e 825 (M + Na), negative ion m/e 801 (M - H)] and gel filtration (Sephadex G-15, in 50% aqueous acetic acid; see later text) and was consistent with the structure suggested by ^{1}H NMR (360 MHz). The later eluting peak isolated by HPLC, X, was characterized as the dimeric material (B) by both mass spectrometry [FAB positive ion m/e 1628 (M + Na)] and gel filtration (Sephadex G-15, in 50% aqueous acetic acid) and was consistent with the structure suggested by ^{1}H NMR (360 MHz). – [illegible]

Cyclo(Gly-Gln-Trp-Phe-(*R*)-Gly[ANC]Leu-Met) (XVI) (Procedure A). The preparation of the protected peptide resin 8p was similar to that described earlier except that Fmoc was used to protect the final residue (instead of Boc) and this terminal protecting group was removed prior to removal of the peptide from the solid-phase resin to give peptide hydrazide 10p. Thus resin-bound protected peptide Fmoc-Trp-Phe-(*R*)-Gly[ANC-2]-Leu-Met (0.48 mmol, obtained during the preparation of I was deprotected (wash cycle B) and acylated using Fmoc-Gln-ONp in the presence of HOBt in DMF (20 mL) for 60 min. After deprotection of the resin (wash cycle B) it was acylated using the preformed symmetrical anhydride of Fmoc-Gly (2.85 mmol) for 1 h and finally deprotected (wash cycle B). The deprotected peptide was cleaved from the resin with hydrazine hydrate (5% in methanol; 50 mL) for 16 h. The solution was filtered and exhaustively dried to remove the hydrazine. The crude peptide hydrazide was cyclized (as described earlier and was purified chromatographically by gradient elution on silica gel [E. Merck, Lobar, size C, 4.4 mL/min from chloroform (500 mL) to chloroform/methanol/acetic acid (85:10:5; 500 mL)] to give XVI with estimated purity = 97.5% (HPLC).

Cyclo(Gln-Trp-Phe-Gly-Leu-Met) (XVII). Method 1. The resin-bound peptide was prepared by the sequential coupling of resin 6 with Fmoc-Met, Fmoc-Leu, Fmoc-Gly, Fmoc-Phe, and Fmoc-Trp preformed symmetrical anhydrides and Boc-Gln-ONp active ester in an analogous fashion to that described earlier. The peptide was removed from the resin (5% hydrazine hydrate in methanol, 16 h), deprotected (2.3 M HCl/dioxane, 45 min), and cyclized (as described for I). The crude cyclic peptide was purified using silica gel chromatography under gradient conditions (as described) and reverse-phase chromatography (Zorbax ODS; 9.4 mm × 25 cm; eluting with a linear gradient between 40 and 60% aqueous acetonitrile containing 0.05% TFA at 4 mL/min over 10 min). This gave XVII with an estimated purity > 98% (HPLC): amino acid analysis, Glu, 0.96; Gly, 1.03; Met, 0.80; Leu, 0.99; Phe, 1.02; FAB-MS, positive ion m/e 763 (M + H), negative ion m/e 762 (M - H).

Method 2. (a) Preparation of Resin-Bound Protected Peptide Fmoc-Leu-Met-Gln-Trp-Phe-Gly. Functionalized polydimethylacrylamide resin 6 (1.1 mequiv/g, 5 mmol) was sequentially acylated with $(Fmoc\text{-}Gly)_2O$/NMM/DMAP (1:1:0.1) (30 mmol, 60 min), $(Fmoc\text{-}Phe)_2O$ (15 mmol, 30 min), $(Fmoc\text{-}Trp)_2O$ (10.6 mmol, 40 min), Fmoc-Gln-ONP/HOBt (1:1) (5 mmol, 30 min), $(Fmoc\text{-}Met)_2O$ (15 mmol, 30 min), and $(Fmoc\text{-}Leu)_2O$ (15 mmol, 45 min). The resin was washed with DMF (5 × 1 min) and then divided into two portions (2 and 3 mmol divided by volume). The larger portion (3 mmol) was used below (b).

(b) Deprotection and Cyclization Conditions. A portion of the above resin (3 mmol) was deprotected (wash cycle B) and cleaved by analogy with 8p to give the crude deprotected hexapeptide hydrazide (1.72 g, 2.16 mM) after desiccation over H_2SO_4 and P_2O_5; HPLC, t_R = 13.4 min. This residue was dissolved in DMF (50 mL) and 9.3 M HCl/THF (1.16 mL, 10.8 mmol) and was cyclized as earlier to give the crude cyclic hexapeptide (XVII) (1.25 g). HPLC, t_R = 16.9 min. A sample of this material (50 mg) was purified by repeated reverse-phase chromatography on Lichroprep C-18 (25–40-μm particle size) eluting the product with a linear gradient of H_2O to H_2O/CH_3CN/TFA to yield purified cyclic peptide (XVII) 40 mg.

Molecular Weight Determination by Gel Filtration. The apparent molecular weight was estimated by gel permeation chromatography in 50% aqueous acetic acid with a Sephadex G-15 column (1.6 × 83.5 cm). Peptide fragments structurally related to substance P were used as molecular weight standards and were substance P triacetate (1528), Phe-Phe-Gly-OEt acetate (458), L-363,851[39] (744), phenylalanine acetate (225). The effluent (0.34 mL/min) monitored at 254 nm allowed determination of apparent molecular weight as described by Andrews.[26] The void volume (V_0) was determined by the volume of elution of Dextran Blue. The results of the determination were (peptide, apparent MW, theoretical MW) as follows: II, 575, 692.9; III, 531, 678.8; IV, 569, 692.9; VIII, 638, 789.0; IX, 661, 803.0; X, 1148, 1606.0.

Biological Assays. The biological assays for NK-1, NK-2, and NK-3 have been described previously.[29] NK-1 assays used the isometric contraction of the longitudinal muscle of the small intestine from 300–400-g male Dunkin–Hartley guinea pigs (Bantin and Kingman) in Krebs–Henseleit solution at 37 °C, in the presence of atropine, mepyramine, methysergide (all 5 μM), and indomethacin (1 μM). NK-2 assays used the electrically stimulated contractions of the vas deferens from 200–300-g Sprague–Dawley rats using rectangular bipolar pulses of supramaximal voltage. NK-3 assays used measurement of the frequency and amplitude of the myogenic contractions from longitudinal strips of the portal vein from rats (source as above). In all cases above, the measurement of antagonist activity was derived from the rightward shift of log dose–response curves to eledoisin. Schild regression analysis[41] using three or more observations at each of three or more antagonist concentrations were used to derive pA_2 values. Where solubility or affinity of the test compound were limiting factors, estimates of apparent pA_2 were made at a single concentration of antagonist, from the standard equation for the Schild analysis assuming unit slope.

Acknowledgment. We would like to thank R. H. Herbert and R. Williams for spectral data and L. L. Iversen for help and encouragement.

Supplementary Material Available: C, H, N elemental analysis of 3 and 5 and ^{1}H NMR data for I–VII, X, XI, XIII, XIV, and XVI (3 pages). Ordering information is given on any current masthead.

(41) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* 1959, *14*, 48–58.

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COMMUNICATION

The European Patent Office herewith transmits

- ☐ the European search report
- ☐ the declaration under Rule 45 EPC
- ☒ the partial European search report under Rule 45 EPC
- ☐ the supplementary European search report concerning the international application under Article 157(2) EPC relating to the above-mentioned European patent application. Copies of the documents cited in the search report are enclosed.

The following specifications given by the applicant have been approved by the Search Division :

☒ Abstract ☒ Title ☐ Figure

- ☐ The abstract was modified by the Search Division and the definitive text is attached to this communication.
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  Figure:

- ☒ Additional copy(copies) of the documents cited in the European search report.

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Application Number EP 98 20 4483

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	BRIAN J. WILLIAMS ET AL.: "Cyclic peptides as selective tachykinin antagonists." JOURNAL OF MEDICINAL CHEMISTRY., vol. 36, no. 1, - 8 January 1993 AMERICAN CHEMICAL SOCIETY. WASHINGTON., US, pages 2-10, XP002114220 * page 7, text part *	1,18	C07D215/52 A61K31/47 C07D409/04 C07D405/04 C07D401/04 C07D409/12 C07D221/18 C07D417/04 C07D401/12 C07D405/12

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07D
A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

Although claims 21 and 22 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Place of search	Date of completion of the search	Examiner
THE HAGUE	6 September 1999	Van Bijlen, H

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